Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

ON THE PREPARATION OF CHICK EMBRYOS FOR MICROSCOPICAL EXAMINATION.

W. P. Manton, M. D., Detroit, Mich.

The embryology of the domestic fowl, *Gallus domesticus*, offers at once one of the most fascinating and instructive studies within the reach of every possessor of a microscope.

Eggs can be procured at all times, and the reagents, instruments, &c., with perhaps one or two exceptions, necessary to carry on the investigations, are inexpensive and easily obtained.

Very little attention has been paid by the general microscopist to this branch of research ; partly, perhaps, because the books on the practical side of the subject are few and expensive. I therefore offer the following brief directions for preparing chick embryos for microscopical examination, with the hope that they may be of service to the beginner, and awaken an interest in this subject.

The necessary reagents are: A 1 per cent. solution of sodium chloride; a 3 per cent. solution of chromic acid; borax carmine; alcohol 70 per cent., 95 per cent. and absolute (Squibs) strength; hydro-chloric acid; spirits of chloroform; oil of turpentine; oil of cloves; Canada balsam; a solution of white shellac in absolute alcohol, rather thinner than glycerine; and paraffine of highest and lowest melting point.

The picric acid solution may be prepared thus: Make a saturated solution of the acid with 100 parts of cold water; to this add two parts of concentrated sulphuric acid; filter, and add to the filtrate three times its bulk of water. To prepare the borax carmine, take 1 pint of 70 per cent. alcohol; one pint of distilled water; three drachms of carmine; and five drachms of borax. These are mixed and cooked over a water bath, and, when cool, filtered.

The easiest way of obtaining embryos for examination is to place eggs, marked with the *date* and *hour*, under a sitting hen, removing one or more at stated intervals, hardening and placing the specimens in 95 per cent. alcohol for future consideration. The date, &c., should be written on the shell with ink; and it should lie uppermost so that it can be readily seen without disturbing the eggs.

The first egg should be removed after about eighteen hours of incubation, when the first two layers of the blastoderm are well differentiated. After thirty-six hours, the three layers—epi-, mero- and hypoblast,—may be seen. The eggs of forty-eight hours, as well as those of three, four and five days' incubation, should also be treated in the same manner. As soon as one egg is removed from the nest another may be put in its place, and thus, in the course of a week or ten days, a sufficient number of embryos may be obtained to furnish work for the odd hours during many winter months. Older embryos, up to the eighth day, should also be obtained for the study of the completely-formed organs, &c.

The egg to be opened should be carefully removed from under the hen, with the side on which the date is written uppermost, for the embryo always lies on top of the yolk. The egg is then rested on a glass individual salt-cellar, and the shell broken at the larger end to let in air. Then the egg and the cellar should be transferred to a dish of the salt solution which has been warmed to blood heat. The depth of the salt water should be sufficient to quite cover the entire egg. The upper part of the shell must now be broken by a few taps of the scissors or scalpel handle, and the pieces removed with fine forceps over the space the size of a quarter of a dollar. This requires some little care, as the sharp-pointed bits of shell are apt to turn inward and cut the embryo, or open the yolk. When this has been accomplished the embryo will be seen lying within two rings, an opaque one, the *area opaca*, and a transparent one, the *area pellucida*. A circling cut must now be made around these rings with a pair of fine-pointed, curved scissors,—those used by the oculist are the best,—and the disk containing the embryo floated off and washed free of any adherent yolk in the salt solution. While this is going on we must provide a small dish such as a glass sauce dish, with a layer of wax at the bottom. This is quickly done by pouring melted beeswax into the dish and allowing it to spread out evenly. I have already indicated two hardening fluids,—picric acid and chromic acid. After some little experience with both, I have come to prefer the former, and for two reasons. It renders the specimens less brittle, and they take the stain much better. Therefore, as the chromic acid method is much inferior to the picric acid, I shall give the latter only.

The saucer containing the wax is now filled with the Kleinberg's solution, and the embryo transferred to it on a spatula from the salt water. The edges of the embryonic disc must then be drawn out and pinned down, so that the embryo will harden without wrinkles. For this purpose I like the long, German insect pins, as they are easy to handle and are not readily affected by the acid.

The embryo should be left to harden undisturbed for from five to twenty four hours, according to size. When this is done, prepare a glass jar,—a half-pint fruit-jar or a tumbler will answer for this purpose,—with 70 per cent. alcohol, in which a piece of cotton floats just below the surface. Upon this cotton lay the embryo, and allow it to remain until the alcohol has withdrawn the picric acid, and the specimen has become whitened. It is frequently necessary to change the alcohol several times before this is accomplished. When the embryo is quite bleached it may be placed in 95 per cent. alcohol for indefinite keeping.

If it is desired to stain at once, the embryo should be placed from the 70 per cent. alcohol into water, and, when washed, into the borax carmine, where it may remain for several hours. It should then be washed again in 70 per cent. alcohol to which a drop of concentrated hydrochloric acid has been added.

After staining, &c., the embryo should be placed in absolute alcohol for several hours. From this soak in chloroform for about half an hour, and then place in a watch-glass containing chloroform and shavings of paraffine. This watch-glass must then be slowly heated, either in a drying-box kept at about 113° F. or in a water bath, until the paraffine is melted and the chloroform driven off. This is done in order that all traces of alcohol and chloroform may be removed, and the embryo thoroughly permeated with the paraffine.

The embryo is now to be placed at one end of a paper cell which has previously been partly filled with melted paraffine and the latter allowed to cool, and covered with warm paraffine. Should the specimen float out of place it may be returned to any position by the point of a hot needle. And I may say here, that in all manipulations after the embryo has come in contact with the paraffine, the spatulas, needles, &c., used should be kept warm by holding in the spirit-lamp flame; otherwise the specimens will adhere to them and perhaps become torn and ruined. Air-bubbles, which frequently collect about the specimen, should be displaced by a hot needle before the upper layer of paraffine has become hardened.

Before proceeding to cut sections of the embedded embryo, it is necessary to have a sufficient number of glass slides in readiness. These should be perfectly clean and clear. Each in turn must then be warmed over the spirit-lamp flame, and when the mist which collects on the application of the heat, disappears, a glass rod dipped in the shellac solution (already described), must be pushed over the slide in such a way as to leave behind it a thin film of the shellac. If this film turns opaque it indicates that the slide was not hot enough, and in that condition is unfit for use. The film should be perfectly smooth, free from waves, and so thin as to be hardly perceptible. When ready to make sections a slide should be selected, and a thin coating of clove oil given it with a camel's-hair brush, over that portion covered with the shellac film. Care should be taken not to go over the same spot twice, as the oil dissolves the varnish.

A microtome is almost indispensable for cutting embryos; but one used to free-hand cutting with a razor may make very good sections, but with infinitely more labor. The paraffine should be trimmed down to within a few lines of the specimen, and each consecutive section laid on the prepared slide, beginning at the upper right-hand corner and continuing in rows from right to left. In this manner I have been enabled to place 126 sections in good order under a cover glass less than an inch square. The clove oil will cause the sections to adhere to the slide, so that once in place, they remain. When a sufficient number of sections has been placed, the slide should be carefully heated over the lamp flame. This melts the shellac film, permanently fixing the sections. The paraffine is also liquefied, and by placing the slide on the edge it may be collected to one point. When the slide has cooled, a few drops of oil of turpentine should be poured over the sections to dissolve the paraffine. The turpentine should then be run off, and the glass carefully wiped up to the sections. A drop of Canada balsam and the adjusting of the cover glass concludes the process.

The finished specimen should be labeled, and if a series is prepared, the number of slide and series may be placed at the lower right-hand corner of the label.

Although the above process is apparently complicated, it is really quite simple and easy, and, if carefully carried out, fully repays whatever pains have been taken in its execution.